REMARKS

Claim Rejections Under 35 USC § 112

In the Office Action dated April 7, 2004, the Examiner rejected claims 22-42 under 35 USC 112, ¶1, as failing to comply with the written description requirement.

In the 35 U.S.C. 112, ¶1 rejection, the Examiner stated that the claims were amended on January 15, 2004, without pointing out where in the specification the amendments to the claims find support.

Applicants respectfully submit that the claims as now amended fully find support in the paragraphs of the Specification as cited herein below.

Regarding the amendments to the claims as currently pending that recite the steps of "obtaining gDNA from a source wherein a portion of the gDNA encodes a desired individual MHC heavy chain molecule" and "creating a PCR product encoding a soluble form of the desired MHC heavy chain molecule by PCR amplification of the gDNA, wherein the amplification utilizes at least one locus-specific primer having a stop codon incorporated into a 3' primer thereby resulting in a PCR product that does not encode the cytoplasmic and transmembrane domains of the desired MHC heavy chain molecule, thereby producing a PCR product that encodes a soluble MHC heavy chain molecule", support for such limitations can be found in the following paragraphs:

(1) Paragraph [0203] of the Specification as originally filed expressly discloses that the "method of the present invention *begins by obtaining*

genomic DNA which encodes the desired MHC class I or class II molecule. Alleles at the locus which encode the desired MHC molecule are *PCR amplified* in a locus specific manner. These locus specific PCR products may include the entire coding region of the MHC molecule or a portion thereof. In some cases a nested or hemi-nested PCR is applied to produce *a truncated form of the class I or class II gene so that it will be secreted rather than anchored to the cell surface*. In other cases the PCR will directly truncate the MHC molecule."

(2) Paragraph [0345] of the Specification as originally filed expressly discloses that "[i]n the recombinant truncated HLA class I molecule (*soluble HLA or sHLA*, approximately 47 kDa), the *C-terminal intracellular and transmembrane domains of the heavy chain are not amplified by PCR and thus deleted*, resulting in a functional MHC/peptide complex that is secreted from the cell and still capable of interaction with the TCR."

(3) Paragraph [0119] of the Specification as originally filed expressly discloses that "[t]he present invention envisions a method of producing MHC molecules which are secreted from mammalian cells in a bioreactor unit. Substantial quantities of individual MHC molecules are obtained by modifying class I or class II molecules so they are secreted. *Secretion of soluble MHC molecules* overcomes the disadvantages and defects of the prior art in relation to the quantity and purity of MHC molecules produced."

(4) Paragraph [0125] of the Specification as originally filed expressly discloses that the "PCR products contained the leader peptide, α_1, α_2, and α_3 coding domains of the HLA heavy chain", while FIG. 1 illustrates a map of the MHC heavy chain coding region including these domains, and FIG. 29 contains a graphical representation of the PCR strategy for the production of soluble HLA from gDNA according to the methods of the present invention.

(5) Paragraph [0206] of the Specification as originally filed expressly discloses that "in the method of the present invention, only a saliva *sample*, a hair root, an old freezer *sample*, or less than a milliliter (0.2 ml) of blood would be required to isolate the gDNA".

(6) Paragraph [0212] of the Specification as originally filed expressly discloses that "[g]reater than or equal to 200ul of *sample* either blood, plasma, serum, buffy coat, body fluid or up to $5x10^6$ lymphocytes in 200ul phosphate buffered saline was used to extract genomic DNA using the QIAampr DNA Blood Mini Kit blood and body fluid spin protocol".

Regarding the amendments to the claims as currently pending that recite the step of "inoculating the cell pharm with the at least one suitable host cell containing the plasmid such that the cell pharm produces soluble MHC complexes having the desired MHC heavy chain molecule associated with native beta-2-microglobulin and loaded with endogenously produced peptides", support for such limitations can be found in the paragraphs cited above as well as in the following paragraphs:

(1) Paragraph [0011] of the Specification as originally filed expressly discloses that "[t]he heavy chains of class I molecules are encoded within the MHC and, upon assembling into heterodimers with the light chain, β_2m, are responsible for selectively gathering endogenously processed peptides. Once peptides are collected, mature class I molecules transport the bound peptides to the cell surface where receptors on $CD8^+$ T lymphocytes engage the class I molecules to inspect the ligands". Fig. 4 illustrates this process.

(2) Paragraphs [0344-0347] of the Specification as originally filed expressly disclose that:

[0344] Structurally, class I molecules are *heterodimers* comprised of two noncovalently bound polypeptide chains, *a larger "heavy" chain (α) and a smaller "light" chain (β-2-microglobulin, or β_2m)*. The polymorphic, polygenic heavy chain (45 kDa), encoded within the MHC on chromosome six, is subdivided into three extracellular domains (designated α_1, α_2, and α_3), one intracellular domain, and one transmembrane domain (FIG. 36). The two outermost extracellular domains, α_1 and α_2, together form the groove that binds antigenic peptide (FIG. 37). Thus, interaction with the TCR occurs at this region of the protein. The α_3 domain of the molecule contains the recognition site for the CD8 protein on the CTL; this interaction serves to stabilize the contact between the T cell and the APC.

[0345] *The invariant light chain (12 kDa), encoded outside the MHC on chromosome 15, consists of a single, extracellular polypeptide.* In the recombinant truncated HLA class I molecule (soluble HLA or sHLA, approximately 47 kDa), the C-terminal intracellular and transmembrane domains of the heavy chain are not amplified by PCR and thus deleted, resulting in a functional MHC/peptide complex that is secreted from the cell and still capable of interaction with the TCR.

[0346] Antigen processing and assembly of the MHC class I/peptide complex. The heavy chain of the class I heterodimer is cotranslationally inserted into the lumen of the ER (FIG. 38). The extracellular (intralumenal) domains of newly synthesized α chains are glycosylated and become immediately associated with the ER chaperone protein calnexin. Calnexin is a membrane-bound molecule that functions to temporarily keep the heavy chain in a partially folded state. The subsequent noncovalent interaction of free β_2m with the heavy chain causes the release of calnexin, and the *heavy chain/β_2m complex* becomes sequentially associated with the chaperones calreticulin and tapasin. *These chaperones position the class I heterodimer in a manner that enables it to be loaded with the processed peptides* once they are transported into the ER by the TAP (transporter associated with antigen processing) complex, located within the ER membrane.

[0347] In the cytosol, a multisubunit, multicatalytic protease complex called the proteosome functions to prepare the endogenously synthesized proteins for presentation by MHC class I molecules. Once proteins (viral or otherwise) are cleaved into peptides of various lengths by the proteosome, the TAP complex actively transports the peptides into the ER where the MHC heterodimer awaits. In the ER lumen, peptides are further trimmed to lengths of 8-11 amino acids. If possessing the requisite binding affinity to the particular class I allele, *a peptide will be loaded into the binding cleft of the class I heavy chain to form the MHC heavy chain/light chain/peptide heterotrimeric complex*, which is subsequently routed to the cell surface via the Golgi apparatus (FIG. 39).

(3) FIGs. 36, 38 and 39 illustrate the process of complexing the desired MHC heavy chain molecule with native beta-2-microglobulin and loading with endogenously produced peptide.

Regarding the amendments to the claims as currently pending that recite the step of "isolating mRNA from the gDNA and reverse transcribing the mRNA to obtain cDNA, wherein the cDNA contains cDNA encoding the desired MHC heavy chain molecule", support for such limitations can be found in the following paragraphs:

(1) Paragraph [0205] of the Specification as originally filed expressly disclose that: "Cloned genomic DNA fragments contain both exons and introns as well as other non-translated regions at the 5' and 3' termini of the gene.

Following transfection into a cell line which transcribes the genomic DNA (gDNA) into RNA, cloned genomic DNA results in a protein product thereby removing introns and splicing the RNA to form messenger RNA (mRNA), which is then translated into an MHC protein. Transfection of MHC molecules encoded by gDNA therefore facilitates reisolation of the gDNA, mRNA/cDNA, and protein. Production of MHC molecules in non-mammalian cell lines such as insect and bacterial cells requires cDNA clones, as these lower cell types do not have the ability to splice introns out of RNA transcribed from a gDNA clone. In these instances the mammalian gDNA transfectants of the present invention provide a valuable source of RNA which can be reverse transcribed to form MHC cDNA. The cDNA can then be cloned, transferred into cells, and then translated into protein. In addition to producing secreted MHC, such gDNA transfectants therefore provide a ready source of mRNA, and therefore cDNA clones, which can then be transfected into non-mammalian cells for production of MHC. Thus, the present invention which starts with MHC genomic DNA clones allows for the production of MHC in cells from various species".

(2) Paragraph [0335] of the Specification as originally filed expressly disclose that: "An exemplary useful product which can be obtained from the mammalian cell line expressing such a genomic DNA construct is a cDNA clone encoding the desired class I or class II molecule. The cDNA clone encoding the desired class I or class II molecule is formed from the mRNA molecule encoding the desired class I molecule isolated from such mammalian cell line. The cDNA clone may be utilized for functional testing, as described in more detail herein below. Thus, gDNA clones can be used as a mechanism to obtain cDNA clones of the desired class I or class II HLA molecule".

Therefore, Applicants respectfully submit that the claims as now pending fully comply with the written description requirement of 35 U.S.C. 112, ¶1. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 112, ¶1 rejection of the claims.

In the Office Action dated April 7, 2004, the Examiner rejected claims 22-42 under 35 USC 112, ¶2, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the

invention.

Applicant respectfully submits that the claims, as currently amended, particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Applicant herein has amended the claims to remove the word "source" and replaced it with the word "sample". As such, the rejection under 35 USC 112, ¶2, has been rendered moot and the Examiner is respectfully urged to withdraw this rejection and pass the claims to an expedient issuance.

Conclusion

It is respectfully submitted that this application, as now amended, is in condition for allowance for the reasons stated above. Therefore, it is requested that the Examiner reconsider each and every rejection as applicable to the claims now pending in the application and pass such claims to issue.

This amendment is intended to be a complete response to the Office Action dated April 7, 2004. In the event that any outstanding issues remain that would delay the allowance of this application, the examiner is urged to contact the undersigned to telephonically discuss such outstanding issues.

Respectfully submitted,

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